

What is claimed is:

1. A process for evaluating activity of a nucleic acid cleavage agent present in a sample, the process comprising:
  - a. incubating the sample with a probe, the probe comprising:
    - i. an oligonucleotide that forms a stem loop structure
    - ii. a fluorophore, and
    - iii. a quencher, wherein the fluorophore and the quencher are positioned such that the fluorophore fluoresces less when the probe is intact than when the probe is cleaved;
  - b. measuring the level of fluorescence of the probe; and
  - c. correlating amount of fluorescence with activity of the nucleic acid cleavage agent.
2. The process of claim 1, wherein the nucleic acid cleavage agent is an enzyme.
3. The process of claim 2, wherein the enzyme is a nuclease.
4. The process of claim 3, wherein the nuclease is an exonuclease.
5. The process of claim 3, wherein the nuclease is an endonuclease.
6. The process of claim A5, wherein the enzyme is a restriction endonuclease.
7. The process of claim 1, wherein the nucleic acid cleavage agent is a small molecule.
8. The process of claim 1, wherein the nucleic acid cleavage agent is an enediyne.
9. The process of claim 1, wherein the nucleic acid cleavage agent cleaves the probe in the single stranded portion of the stem loop structure.
10. The process of claim 1, wherein the nucleic acid cleavage agent cleaves the probe in the double stranded portion of the stem loop structure.

11. The process of claim 1 wherein the fluorophore and quencher are internally coupled to the probe.

12. The process of claim 1 wherein the fluorophore and quencher are coupled to the 5' and/ or 3' ends of the probe.

13. The process of claim 1 wherein the nucleic acid cleavage agent cleaves the probe at a site between the quencher and the fluorophore.

14. The process of claim 1 wherein the probe is immobilized to a solid surface.

15. A process for detecting the presence of a nucleic acid cleavage agent in a sample, the process comprising:

a. incubating the sample with a probe, the probe comprising:

i. an oligonucleotide that forms a stem loop structure

ii. a fluorophore, and

iii. a quencher, wherein the fluorophore and the quencher are positioned such that the fluorophore fluoresces less when the probe is intact than when the probe is cleaved; and

b. measuring the level of fluorescence of the probe.

16. The process of claim 15, wherein the nucleic acid cleavage agent is an enzyme.

17. The process of claim 16, wherein the enzyme is a nuclease.

18. The process of claim 17, wherein the nuclease is an exonuclease.

19. The process of claim 17, wherein the nuclease is an endonuclease.

20. The process of claim 19, wherein the enzyme is a restriction endonuclease.

21. The process of claim 15, wherein the nucleic acid cleavage agent is a small molecule.
22. The process of claim 15, wherein the nucleic acid cleavage agent is an enediyne.
23. The process of claim 15, wherein the nucleic acid cleavage agent cleaves the probe in the single stranded portion of the stem loop structure.
24. The process of claim 15, wherein the nucleic acid cleavage agent cleaves the probe in the double stranded portion of the stem loop structure.
25. The process of claim 15 wherein the fluorophore and quencher are internally coupled to the probe.
26. The process of claim 15 wherein the fluorophore and quencher are coupled to the 5' and/ or 3' ends of the probe.
27. The process of claim 15 wherein the nucleic acid cleavage agent cleaves the probe at a site between the quencher and the fluorophore.
28. The process of claim 15 wherein the probe is immobilized to a solid surface.
29. A process for evaluating activity of a nucleic acid cleavage agent present in a sample, the process comprising:
- a. incubating the sample with a probe, the probe comprising:
    - i. an oligonucleotide that forms a stem loop structure and comprises a recognition site for the nucleotide cleavage agent;
    - ii. a fluorophore, and
    - iii. a quencher, wherein the fluorophore and the quencher are positioned such that the fluorophore fluoresces less when the probe is intact than when the probe is cleaved;
  - b. measuring the level of fluorescence of the probe; and

c. correlating amount of fluorescence with activity of the nucleic acid cleavage agent.

30. The process of claim 29, wherein the nucleic acid cleavage agent is an enzyme.

31. The process of claim 30, wherein the enzyme is a nuclease.

32. The process of claim 31, wherein the nuclease is an exonuclease.

33. The process of claim 31, wherein the nuclease is an endonuclease.

34. The process of claim 33, wherein the enzyme is a restriction endonuclease.

35. The process of claim 29, wherein the nucleic acid cleavage agent is a small molecule.

36. The process of claim 29, wherein the nucleic acid cleavage agent is an enediyne.

37. The process of claim 29, wherein the recognition site is located in the single stranded portion of the stem loop structure.

38. The process of claim 29, wherein the recognition site is located in the double stranded portion of the stem loop structure.

39. The process of claim 29, wherein the recognition site spans the junction between the single stranded and the double stranded portions of the stem loop structure.

40. The process of claim 29 wherein the fluorophore and quencher are internally coupled to the probe.

41. The process of claim 29 wherein the fluorophore and quencher are coupled to the 5' and/ or 3' ends of the probe.

42. The process of claim 29 wherein the recognition site is located at a site between the quencher and the fluorophore.
43. The process of claim 29 wherein the probe is immobilized to a solid surface.
44. A process for detecting the presence of a nucleic acid cleavage agent in a sample, the process comprising:
- a. incubating the sample with a probe, the probe comprising:
    - i. an oligonucleotide that forms a stem loop structure and comprises a recognition site for the nucleotide cleavage agent;
    - ii. a fluorophore, and
    - iii. a quencher, wherein the fluorophore and the quencher are positioned such that the fluorophore fluoresces less when the probe is intact than when the probe is cleaved; and
  - b. measuring the level of fluorescence of the probe.
45. The process of claim 44, wherein the nucleic acid cleavage agent is an enzyme.
46. The process of claim 45, wherein the enzyme is a nuclease.
47. The process of claim 46, wherein the nuclease is an exonuclease.
48. The process of claim 46, wherein the nuclease is an endonuclease.
49. The process of claim 48, wherein the enzyme is a restriction endonuclease.
50. The process of claim 44, wherein the nucleic acid cleavage agent is a small molecule.
51. The process of claim 44, wherein the nucleic acid cleavage agent is an enediyne.
52. The process of claim 44, wherein the recognition site is located in the single

stranded portion of the stem loop structure.

53. The process of claim 44, wherein the recognition site is located in the double stranded portion of the stem loop structure.

54. The process of claim 44, wherein the recognition site spans the junction between the single stranded and the double stranded portions of the stem loop structure.

55. The process of claim 44 wherein the fluorophore and quencher are internally coupled to the probe. .

56. The process of claim 44 wherein the fluorophore and quencher are coupled to the 5' and/ or 3' ends of the probe.

57. The process of claim 44 wherein the recognition site is located at a site between the quencher and the fluorophore.

58. The process of claim 44 wherein the probe is immobilized to a solid surface.

59. A process for evaluating activity of a nucleic acid cleavage agent, the process comprising:

a. incubating the nucleotide cleavage agent with a first probe, the first probe comprising:

i. an oligonucleotide that forms a stem loop structure and having a first sequence;

ii. a fluorophore, and

iii. a quencher, wherein the fluorophore and the quencher are positioned such that the fluorophore fluoresces less when the probe is intact than when the probe is cleaved;

b. measuring level of the fluorescence of the first probe;

c. incubating the nucleotide cleavage agent with a second probe, the second probe comprising:

i. an oligonucleotide that forms a stem loop structure and having a second sequence;

- ii. a fluorophore, and
- iii. a quencher, wherein the fluorophore and the quencher are positioned such that the fluorophore does not fluoresce when the probe is intact and does fluoresce when the probe is cleaved;
- d. measuring level of the fluorescence of the second probe;
- e. comparing the level of fluorescence of the first probe to the level of fluorescence of the second probe; and
- f. correlating the amount of fluorescence of the first and second probes with activity of the nucleic acid cleavage agent.

60. The process of claim 59, wherein steps (a) and (c) are carried out in separate reaction vessels.

61. The process of claim 59, wherein steps (a) and (c) are carried out in the same reaction vessel.

62. The process of claim 61, wherein the first probe comprises a first fluorophore and the second probe comprises a second fluorophore, and wherein the first and second fluorophores are distinguishable from one another.

63. The process of claim 59, wherein the nucleic acid cleavage agent is an enzyme.

64. The process of claim 63, wherein the enzyme is a nuclease.

65. The process of claim 64, wherein the nuclease is an exonuclease.

66. The process of claim 64, wherein the nuclease is an endonuclease.

67. The process of claim 66, wherein the enzyme is a restriction endonuclease.

68. The process of claim 59, wherein the nucleic acid cleavage agent is a small molecule.

69. The process of claim 59, wherein the nucleic acid cleavage agent is an enediyne.

70. The process of claim 59, wherein the nucleic acid cleavage agent cleaves the probe in the single stranded portion of the stem loop structure.

71. The process of claim 59, wherein the nucleic acid cleavage agent cleaves the probe in the double stranded portion of the stem loop structure.

72. A process for evaluating activity of a nucleic acid cleavage agent, the process comprising:

a. incubating the nucleotide cleavage agent with a probe in a first set of conditions, the probe comprising:

i. an oligonucleotide that forms a stem loop structure;

ii. a fluorophore, and

iii. a quencher, wherein the fluorophore and the quencher are positioned such that the fluorophore fluoresces less when the probe is intact than when the probe is cleaved;

b. measuring level of fluorescence of the probe in the first set of conditions;

c. incubating the nucleotide cleavage agent with the probe in a second set of conditions;

d. measuring level of fluorescence of the probe in the second set of conditions;

e. comparing the level of fluorescence of the probe in the first set of conditions to the level of fluorescence of the probe in the second set of conditions; and

f. correlating the level of fluorescence in the first and second conditions to the activity of the nucleic acid cleavage agent..

73. The process of claim 72, wherein the nucleic acid cleavage agent is an enzyme.

74. The process of claim 73, wherein the enzyme is a nuclease.

75. The process of claim 74, wherein the nuclease is an exonuclease.



76. The process of claim 74, wherein the nuclease is an endonuclease.
77. The process of claim 76, wherein the enzyme is a restriction endonuclease.
78. The process of claim 72, wherein the nucleic acid cleavage agent is a small molecule.
79. The process of claim 72, wherein the nucleic acid cleavage agent is an enediyne.
80. The process of claim 72, wherein the nucleic acid cleavage agent cleaves the probe in the single stranded portion of the stem loop structure.
81. The process of claim 72, wherein the nucleic acid cleavage agent cleaves the probe in the double stranded portion of the stem loop structure.
82. A process for evaluating the effectiveness of a nucleotide protective agent, the process comprising:
  - a. incubating a nucleotide cleavage agent and a probe, the probe comprising:
    - i. an oligonucleotide that forms a stem loop structure
    - ii. a fluorophore, and
    - iii. a quencher, wherein the fluorophore and the quencher are positioned such that the fluorophore fluoresces less when the probe is intact than when the probe is cleaved;
  - b. measuring the level of fluorescence of the probe as incubated in step (a);
  - c. incubating the nucleotide protective agent, the nucleotide cleavage agent, and the probe;
  - d. measuring the level of fluorescence of the probe as incubated in step (c);
  - e. comparing the levels of fluorescence measured in steps (b) and (d); and
  - f. correlating amount of difference in the fluorescence levels measured in steps (b) and (d) with the effectiveness of the nucleotide protective agent.
83. The process of claim 82, wherein the nucleic acid cleavage agent is an enzyme.

84. The process of claim 83, wherein the enzyme is a nuclease.
85. The process of claim 84, wherein the nuclease is an exonuclease.
86. The process of claim 84, wherein the nuclease is an endonuclease.
87. The process of claim 86, wherein the enzyme is a restriction endonuclease.
88. The process of claim 82, wherein the nucleic acid cleavage agent is a small molecule.
89. The process of claim 82, wherein the nucleic acid cleavage agent is an enediyne.
90. The process of claim 82, wherein the nucleic acid cleavage agent cleaves the probe in the single stranded portion of the stem loop structure.
91. The process of claim 82, wherein the nucleic acid cleavage agent cleaves the probe in the double stranded portion of the stem loop structure.
92. An oligonucleotide probe comprising:
- a. an oligonucleotide that forms a stem loop structure and comprises a recognition site for a nucleotide cleavage agent;
  - b. a fluorophore, and
  - c. a quencher, wherein the fluorophore and the quencher are positioned such that the fluorophore fluoresces less when the probe is intact than when the probe is cleaved.
93. The probe of claim 92, wherein the nucleic acid cleavage agent is an enzyme.
94. The probe of claim 93, wherein the enzyme is a nuclease.

95. The probe of claim 94, wherein the nuclease is an exonuclease.
96. The probe of claim 94, wherein the nuclease is an endonuclease.
97. The probe of claim 96, wherein the enzyme is a restriction endonuclease.
98. The probe of claim 92, wherein the nucleic acid cleavage agent is a small molecule.
99. The probe of claim 92, wherein the nucleic acid cleavage agent is an enediyne.
100. A kit comprising at least one probe according to claim 92.
101. The kit of claim 100, further comprising at least one nucleotide cleavage agent that recognizes the recognition site.
102. The probe of claim 92, wherein the nucleic acid cleavage agent cleaves the probe in the single stranded portion of the stem loop structure.
103. The probe of claim 92, wherein the nucleic acid cleavage agent cleaves the probe in the double stranded portion of the stem loop structure.